

ABSTRACT OF THE DISCLOSURE

A multiplexed enzyme assay method and substrate set for performing the method are disclosed. The method includes performing a plurality of enzyme reactions in the presence of a plurality of enzymes substrates, under conditions effective to convert an
5 enzyme substrate to a corresponding product, where the product of each substrate and the substrate have different separation characteristics from each other and from the other substrates and their corresponding products. After performing the reactions, which may be carried out in separate or combined reactions, the substrates and products in said reactions are separated in a single separation medium. For each separated product and substrate, a
10 separation characteristic effective to identify that product and substrate and a signal related to the amount of the product and substrate is detected. From this, one can determine the amount of substrate converted to the corresponding product in each of the reactions.